FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
220513US0PCT	10/069982

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/08983	13 September 2000	16 September 1999

TITLE OF INVENTION

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE AND AMINO ACIDS

APPLICANT(S) FOR DO/EO/US

Lorena MUGGETTI et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority/ Form PTO-1449
PCT/IB/304/ PCT/IB/308

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/069982	PCT/EP00/08983	220513US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	32 - 20 =	12	x $18.00	**$216.00**	
Independent claims	6 - 3 =	3	x $84.00	**$252.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,358.00**	
Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,358.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,358.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,358.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,358.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

March 14 2002
DATE

220513US-0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

LORENA MUGGETTI ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: NEW U.S. PCT APPLN :
(Based on PCT/EP00/08983)

FILED: HEREWITH :

FOR: FORMULATIONS FOR
PARENTERAL USE OF
ESTRAMUSTINE PHOSPHATE
AND AMINO ACIDS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment to read as follows:

3. (Amended) A formulation according to claim 1 wherein estramustine phosphate is in the form of a pharmaceutically acceptable salt for parenteral use.

6. (Amended) A formulation according to claim 1 comprising estramustine phosphate and arginine in a molar ratio lower than 1:1.

10. (Amended) A formulation according to claim 1 which is a single infusion dosage form comprising at least 1300 mg of the estramustine phosphate.

11. (Amended) A formulation according to claim 1 which is in single infusion dosage form comprising at least 950 mg/m^2 of the estramustine phosphate.

12. (Amended) A formulation according to claim 10 wherein the basic amino acid is arginine.

13. (Amended) A formulation according to claim 1 for intravenous use.

14. (Amended) A formulation according to claim 1 for use in the treatment of cancer.

19. (Amended) A product according to claim 17 wherein the chemotherapeutic agent is selected from the group consisting of taxane derivatives such as paclitaxel and docetaxel; camptothecin and derivatives thereof such as CPT-11 and 9-amino-camptothecin; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin, alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives such as carboplatin and cisplatin; angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668; optionally within liposomal formulations thereof.

Please add the following new claim:

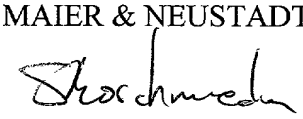
32. (New) The formulation according to Claim 11 wherein the basic amino acid is arginine.

REMARKS

Claims 1-32 are active in the present application. Claims 3, 6, 10-14 and 19 have been amended to remove multiple dependencies. Claim 32 is a new claim. Support for the new claim is found in the original claims. No new matter is added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Stefan U. Koschmieder, Ph.D.
Registration No. 50,238

22850

(703) 413-3000
Fax #: (703)413-2220
NFO/kst

I:\atty\SUKOS\220513us-pr.wpd

Marked-Up Copy
Serial No:

Amendment Filed on:
3-14-2002

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment to read as follows:

--3. (Amended) A formulation according to claim 1 [or 2] wherein estramustine phosphate is in the form of a pharmaceutically acceptable salt for parenteral use.

6. (Amended) A formulation according to [anyone of the preceding claims] claim 1 comprising estramustine phosphate and arginine in a molar ratio lower than 1:1.

10. (Amended) A formulation according to [any one of the preceding claims] claim 1 which is a single infusion dosage form comprising at least 1300 mg of the estramustine phosphate.

11. (Amended) A formulation according to [any one of the preceding claims] claim 1 which is in single infusion dosage form comprising at least 950 mg/m^2 of the estramustine phosphate.

12. (Amended) A formulation according to claim 10 [or 11] wherein the basic amino acid is arginine.

13. (Amended) A formulation according to [any one of the preceding claims] claim 1 for intravenous use.

14. (Amended) A formulation according to [any one of the preceding claims] claim 1 for use in the treatment of cancer.

19. (Amended) A product according to claim 17 [or 18] wherein the chemotherapeutic agent is selected from the group consisting of taxane derivatives such as paclitaxel and docetaxel; camptothecin and derivatives thereof such as CPT-11 and 9-amino-camptothecin; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin, alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives such as carboplatin and cisplatin; angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668; optionally within liposomal formulations thereof.

Claim 32 (New).--

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE AND AMINO ACIDS

The present invention relates to pharmaceutical formulations of estramustine phosphate for parenteral use and, more particularly, to formulations of estramustine phosphate for parenteral use further comprising basic amino acids.

Estramustine phosphate (The Merck Index, XII Ed., No. 3749, 1996) is an estradiol-17β-phosphate derivative widely known in the art as antitumor agent, currently used in the treatment of advanced adenocarcinoma of the prostate.
The drug is usually administered orally, preferably at a dose of 10-15 mg/kg/day. Intravenous administration, however, is also adopted in some particular cases.
For example, initial intravenous administration of estramustine phosphate, followed by oral administration, has been reported at dosages paralleling the oral administration for the drug, i.e. 300-600 mg daily given intravenously and usually repetitively over for several consecutive days (see, for a reference, British Journal of Urology, 1977, 49, 73-79; J. Urol.108:303-306, 1972; Eur. Clin. Pharmacol. 26(1), 113-119, 1984; Eur. Urol. 1990, 17, 216-218).

Estramustine phosphate as well as other well-known cytotoxic compounds used in antitumor therapy are known to cause, or potentially cause, vascular damages at the site of injection when parenterally, in particular intravenously, administered.
As an example, studies in patients treated with estramustine phosphate administered as a slow intravenous injection or as a bolus, at 300 mg/day, revealed

thrombophlebitis and local irritations at the peripheral intravenous injection sites.
These drawbacks are considered major limitations for the intravenous administration of estramustine phosphate, thus requiring, in many patients, the establishment of central line administration or, in some cases, even discontinuation of the treatment.

With the aim of minimising the unwanted effects associated with the intravenous administration of cytotoxic agents, a few means are reported in the art.
Among them is the use of cyclodextrins, for instance hydroxypropyl-cyclodextrin, in the preparation of formulations for parenteral administration of cytotoxic known to cause ulcerative lesions. See, for a reference, US patent No. 5,804,568 in the name of Supergen Inc.
Also known in the art are formulations for the intravenous administration of estramustine phosphate containing human albumin, reported to be characterised by fewer local side-effects upon injection of the active (see, for a reference, H. Schutz et al.; Krankenhauspharmazie, II year, issue No. 3, 1988).

In this respect, we found formulations for parenteral use comprising estramustine phosphate together with a basic amino acid which, unexpectedly, resulted to achieve optimal protection from side-effects associated with estramustine administration.

It is therefore the object of the present invention a formulation for parenteral use comprising estramustine phosphate and a basic amino acid.

Once administered intravenously to patients, the formulations object of the present invention do not provoke ulcerative damages, nor thrombophlebitis, at the site of injection.

In the present description, with the term basic amino acid we preferably intend a basic α-amino acid; preferred basic amino acids are selected from the group consisting of arginine, histidine and lysine.

Even more preferably, the formulations object of the present invention comprise arginine.

With the term formulation comprising estramustine phosphate we intend any formulation of estramustine phosphate, as the active ingredient, either in the acid form or as a pharmaceutically acceptable salt for parenteral use such as, for instance, the salts of estramustine phosphate with basic amino acids themselves or with N-methyl glucamine, otherwise referred to as meglumine.

Preferably, the formulations of the invention comprise estramustine phosphate in the form of its arginine or meglumine salt.

From the foregoing, it is clear to the man skilled in the art that the formulations of the present invention may contain the basic amino acid, either in the form of its salt with estramustine phosphate as well as in admixture with an estramustine phosphate salt for parenteral use.

According to a preferred embodiment of the invention, it is herewith provided a formulation for parenteral use comprising estramustine phosphate, in the form of its arginine salt, optionally in admixture with additional amounts of a basic amino acid, e.g. arginine.

Another preferred embodiment of the invention is a formulation for parenteral use comprising estramustine phosphate, in the form of its meglumine salt, in admixture with arginine.

In another preferred embodiment, estramustine phosphate is provided in lyophilised form and the basic amino acid is

provided in physiological solution. Formulations of this type may typically be provided as a kit.

The formulations object of the present invention may further contain additional excipients for parenteral use. Among them are also those excipients known in the art to reduce the side effects associated with the parenteral administration of cytotoxic agents (herewith referred to as protective agents) such as, for instance, human albumin, cyclodextrins, sulfoalkyl ether cyclodextrins and derivatives thereof.
Preferably, the formulations of the invention additionally comprise human albumin.

Very interestingly, the formulations of the invention also containing a given amount of human albumin are unexpectedly less toxic than the corresponding estramustine phosphate formulations containing the same amount of human albumin, as the sole protective agent against ulcerative damages and thrombophlebitis at the site of injection.

It is therefore a further object of the invention a formulation for parenteral use comprising estramustine phosphate and a basic amino acid in admixture with human albumin.

All of the above formulations are preferably intended for intravenous use. As such, they can be administered to patients either as a slow injection, e.g. over about 30 minutes to about 3 hours, or as a bolus injection, also referred to as IV (intravenous) push.

In addition, the formulations of the invention provide a very advantageous method for delivering estramustine phosphate intravenously, even when high doses of the active are needed.

It is therefore a further object of the invention a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 1300 mg, and a basic amino acid.

According to another preferred embodiment of the invention, it is further provided a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 950 mg/m^2, and a basic amino acid.

The formulations of the invention allow the administration of the active either as a single agent or, alternatively, according to a combined chemotherapy regimen.

As an example, the above formulations can be administered in combination with one or more chemotherapeutic agents such as, for instance, taxane derivatives, e.g. paclitaxel and docetaxel; camptothecin and derivatives thereof, e.g. CPT-11, 9-amino-camptothecin; anthracycline derivatives, e.g. doxorubicin, epirubicin, idarubicin, daunorubicin, alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives, e.g. carboplatin and cisplatin; angiogenesis inhibitors, e.g. Sugen SU-5416 and Sugen SU-6668; and the like, optionally within liposomal formulations thereof.

Therefore, it is a further object of the present invention a product containing a formulation for parenteral use comprising estramustine phosphate and a basic amino acid, preferably arginine, and one or more chemotherapeutic agents, as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

Toxicology

To study the local irritant effects of estramustine phosphate (hereinafter referred to as EMP) after repeated intravenous administrations to rats, the active was

prepared, according to the present invention, either in the form of a salt with a basic amino acid and/or in admixture with a basic amino acid. Subsequently, the said preparations were dissolved in different vehicles such as water solution for injection and water solution for injection further containing human albumin (hereinafter referred to as HA). In addition, formulations of estramustine phosphate in the form of meglumine (hereinafter referred to as MEG) salts, optionally in admixture with HA only, were used for comparison.

In particular, the following water for injection solutions (Table I) wherein either the active EMP as well as the basic amino acid or HA are expressed in terms of molar or weight ratios, were prepared and tested:

Table I

Solution	**Molar ratio (mol/mol)**	**Weight ratio (w/w)**
a)negative control water for inject.	-	-
b)positive control EMP-MEG salt	EMP:MEG=1:1	-
c)comparison EMP-MEG salt + HA	EMP:MEG=1:1	EMP:HA=1:0.21
d) EMP-Arg salt	EMP:Arg=1:1	
e) EMP-Arg salt + Arg	EMP:Arg=1:2	
f) EMP-Arg salt + HA	EMP:Arg=1:1	EMP:HA=1:0.21
g) EMP-MEG salt + Arg	EMP:MEG:Arg=1:1:2	

Male Sprague-Dawley rats were used because of their acceptance as a predictor of toxic change in man. The rats were 6 weeks old at the start of the study.

The above formulations were administered to groups of rats as a repeated intravenous injection during 3 days. Rats were then sacrificed: a half of the rats at the fourth day and a half at the fifth day.

The dose level of estramustine phosphate, in all the different tested solutions, was of 150 mg/kg/day.

Clinical observations were recorded daily. Thrombophlebitic side effects resulted in a dark bluish/blackish coloration of the tail during the treatment period.

A score system based on tail coloration and its extension was used to evaluate the different tested formulations.

The score system considered the formulation of estramustine phosphate salt with meglumine, solution **(b)**, as the positive control (i.e. marked toxicity). Water for injection **(a)** was administered to the control group as negative control (i.e. no toxicity signs).

Histological evaluation was carried out on the tail of the rats treated with the composition of the invention.

Estramustine phosphate in a water solution **(b)** induced, at the used dose, local irritant effects at the injection site after the first administration and marked toxicity signs at the end of the experiment.

Likewise, toxicity signs at the injection site were also observed for the comparison **(c)** EMP solution containing human albumin, as the sole protective excipient against ulcerative damages at the injection site.

On the contrary, a markedly reduced toxicity or even no toxicity was observed with the formulations of the invention containing arginine **(d)**, **(e)** and **(g)**, optionally in admixture with human albumin **(f)**.

The histological evaluation of the tail of the rats treated with these formulations confirmed the above findings.

In addition, the protective effect exerted by the basic amino acid, according to the invention, can clearly be evidenced by considering the formulation **(f)** in comparison to the formulations **(c)** for which toxicity signs were observed. Very interestingly, in fact, both formulations

contained the same amount of human albumin with respect to the active.

It was thus concluded that estramustine phosphate in a water solution containing a basic amino acid, either in the form of a salt with estramustine phosphate or in admixture with the same, induced markedly less local irritant effects when compared with a water solution of estramustine phosphate itself.

Even more surprisingly, the formulations of the invention produced less local irritant effects also in comparison to analogous solutions of estramustine phosphate containing known protective excipients, for instance human albumin.

One particularly preferred schedule for administering the formulation of estramustine phosphate according to the invention is a single infusion given once weekly to a maximal dose of 4000 mg or 3500 mg/m^2.

Another preferred schedule is the administration of a single drug infusion once every two to four weeks.

One schedule may be preferred over another in consideration of schedules with other optional concomitant therapy. These schedules may repeat in serial or as repetitive fashion.

The formulations of the present invention are useful in antitumor therapy, particularly in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

In addition to the above, we also noticed that arginine and the pharmaceutically acceptable salts thereof for parenteral use, exerted their protective effects against the occurrence of thrombophlebitis also when used in combination with several other antineoplastic agents known to cause, or potentially cause, ulcerative damages at the site of injection, when administered intravenously.

It is therefore a further object of the invention a pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, an antineoplastic agent known to cause ulcerative damages at the site of injection upon intravenous administration, and arginine or a pharmaceutically acceptable salt thereof.

Besides estramustine phosphate, other antineoplastic agents are known to cause, at least potentially, ulcerative damages and thrombophlebitis at the site of injection. Preferably, these antineoplastic agents according to the invention are selected from the group consisting of anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

From the foregoing, it is a further object of the invention the use of arginine, or pharmaceutically acceptable salts thereof, in the preparation of a medicament for the treatment and prevention of side-effects associated with the intravenous administration of antineoplastic agents.

As set forth above, these side-effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

In the present description, unless otherwise specified, with the term pharmaceutically acceptable salt of arginine, we intend any salt for parenteral use including, preferably, the arginine addition salt with hydrochloric, glutamic or aspartic acid.

The formulations object of the present invention are prepared according to conventional techniques adopted in the preparation of pharmaceutical forms for parenteral use.

Typically, a proper amount of estramustine phosphate is dispersed in water and then dissolved by adding at least an equimolar amount of a basic amino acid, for instance arginine.

A further amount of the given amino acid, e.g. arginine, can be present in order to reach an estramustine phosphate:arginine molar ratio higher than 1:1, respectively.

Alternatively, a proper amount of estramustine phosphate in the form of a pharmaceutically acceptable salt for parenteral use, e.g. estramustine phosphate meglumine salt, either as a dry powder or into a lyophilised form, is dissolved in a pharmaceutically acceptable solution for parenteral use, for instance sterile water or aqueous dextrose solution, e.g. 5% dextrose in water for intravenous administration, and then admixed with a proper amount of a basic amino acid, for instance arginine.

The above admixture is then stirred, sterilised, and subsequently lyophilised according to conventional techniques.

The freeze-dried formulation is prepared and stored in vials for injection; the addition of a proper amount of sterile water or a physiological solution for parenteral use enables the preparation of the final formulation to be injected.

The above method is also suitable for preparing high dosages estramustine phosphate formulations whilst maintaining the desired weight or molar ratio between the components.

The unit strength of the formulation to be injected depended on the concentration of the active in the solution itself and, of course, on the filling volume of the vials used to prepare the final formulation.

Additionally, besides the aforementioned human albumin, cyclodextrins, sulfoalkyl ether cyclodextrins and

derivatives thereof, the formulations of the invention may optionally contain additional pharmaceutically acceptable excipients for parenteral administration such as, for instance, bulking agents, e.g. lactose or mannitol, pH buffering agents, anti-oxidant agents, preservative agents, tonicity adjusters and the like.

From the foregoing, it is clear to the man skilled in the art that the above methods for preparing the estramustine formulations apply as well to arginine-containing formulations comprising antineoplastic agents other than estramustine phosphate, and known to cause, or potentially cause, thrombophlebitic side-effects upon intravenous administration.

The following examples are herewith intended to better illustrate the present invention without representing any limitation to it.

Example 1

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with human albumin (estramustine phosphate:meglumine=1:1 molar ratio, estramustine phosphate:albumin=1:0.21 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 0.250 ml of a commercially available solution of human albumin at 25% concentration were added whilst maintaining the solution under stirring.

The obtained solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 2

The formulation described in Example 1 was also prepared by dissolving the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a 6.25 mg/ml human albumin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

The albumin solution could be prepared either by dissolving in water a proper amount of human albumin as a dry powder or by properly diluting a commercially available human albumin solution.

Example 3

Preparation of estramustine phosphate arginine salt (estramustine phosphate:arginine=1:1 molar ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 101 mg of arginine base were then added to the watery dispersion of the active whilst maintaining under stirring until a clear solution was obtained.

The prepared solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 10.1 mg/ml of arginine (1:1 molar ratio respectively).

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 4

Preparation of estramustine phosphate arginine salt in admixture with arginine (estramustine phosphate:arginine=1:2 molar ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 202 mg of arginine base were then added to the watery dispersion of the active whilst maintaining under stirring until a clear solution was obtained. The basic pH of the obtained solution was brought to the physiological value of about 7.5 by slow addition of diluted hydrochloric acid.

The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 20.2 mg/ml of arginine (1:2 molar ratio respectively).

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 5

Preparation of estramustine phosphate arginine salt in admixture with human albumin (estramustine phosphate:arginine=1:1 molar ratio, estramustine phosphate:albumin=1:0.21 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 101 mg of arginine base were then added to the watery dispersion of the active whilst maintaining under stirring until a clear solution was obtained. 0.250 ml of a commercially available solution of human albumin at 25% concentration were added, maintaining the solution under stirring.

The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate, 10.1 mg/ml of arginine and 6.25 mg/ml of human albumin. The molar and weight ratios between

the components of the solution were as follows: estramustine phosphate:arginine 1:1 molar ratio, estramustine phosphate:human albumin 1:0.21 weight ratio.
A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 6

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with arginine (estramustine phosphate:meglumine:arginine=1:1:2 molar ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. A total amount of 202 mg of arginine was then added to the above described solution by using a suitable mixture of arginine base and hydrochloride, in order to maintain the pH around the physiological value of 7.5.
The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 20.2 mg/ml of arginine (1:2 molar ratio respectively).
A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 7

The formulation described in Example 6 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a 20.2 mg/ml arginine solution so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 20.2 mg/ml of arginine (1:2 molar ratio respectively).

The arginine solution used to dissolve the freeze-dried formulation contained a proper mixture of arginine base and hydrochloride, in order to maintain the pH around the physiological value of 7.5.

Example 8

Preparation of a formulation comprising doxorubicin and arginine in a 1:1 molar ratio.

Doxorubicin hydrochloride (40 mg) and arginine (12 mg) were weighed in a flask and the admixture was dissolved in a physiological solution (15 ml) of NaCl 0.9% w/v, under stirring. A solution of hydrochloric acid was then added up to pH = 3. The solution thus prepared was then diluted with the above physiological solution up to a final volume of 20 ml so as to reach a final concentration of 2 mg/ml of doxorubicin and 0.6 mg/ml of arginine (molar ratio 1:1, respectively).

Example 9

Preparation of a formulation comprising doxorubicin and arginine in a 1:2 molar ratio

By working as above described in example 8 and by using an amount of arginine as twice, that is 24 mg of arginine per 40 mg of doxorubicin hydrochloride, a solution comprising doxorubicin and arginine in a molar ratio 1:2, respectively, was thus prepared.

Example 10

Preparation of a formulation comprising Sugen SU-5416 and arginine in a 1:1 molar ratio

10 ml of an aqueous solution of NaCl (0.9% w/v) were diluted with 10 ml of water in a flask so as to obtain a solution of NaCl at 0.45% w/v.

Arginine hydrochloride (39.79 mg) was then added to the resultant solution and dissolved by shaking.

The solution thus prepared was used to dilute a solution of Sugen SU-5416 having the following composition:

Component	Amount % (w/v) in the formulation
Sugen SU 5416	0.45 %
PEG 400	45 %
Benzyl alcohol	2 %
Cremophor EL	31.5 %
Anhydrous Ethanol	Up to 100

The dilution was carried out by admixing one part of the formulation containing the active Sugen SU-5416 with two parts of the solution containing arginine, so as to obtain a final solution of Sugen SU-5416 and arginine in a molar ratio 1:1.

Example 11

Preparation of a formulation comprising Sugen SU-5416 and arginine in a 1:2 molar ratio

By working as above described in example 10 and by using an amount of arginine hydrochloride as twice, that is 79.58 mg, a solution comprising Sugen SU-5416 and arginine in a 1:2 molar ratio, respectively, was thus prepared.

CLAIMS

1. A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent and estramustine phosphate and a basic amino acid.

2. A formulation according to claim 1 wherein the basic amino acid is arginine.

3. A formulation according to claim 1 or 2 wherein estramustine phosphate is in the form of a pharmaceutically acceptable salt for parenteral use.

4. A formulation according to claim 3 wherein the estramustine phosphate is in the form of a salt with arginine, histidine, lysine or N-methyl glucamine.

5. A formulation according to claim 4 wherein the estramustine phosphate is in the form of a salt with arginine or N-methyl glucamine.

6. A formulation according to anyone of the preceding claims comprising estramustine phosphate and arginine in a molar ratio lower than 1:1.

7. A formulation according to claim 6 comprising estramustine phosphate and arginine in a molar ratio of about 1:2.

8. A formulation according to claim 1 which further comprises human albumin, a cyclodextrin or a sulfoalkyl ether cyclodextrin.

9. A formulation according to claim 1 which further comprises human albumin.

10. A formulation according to any one of the preceding claims which is in single infusion dosage form comprising at least 1300 mg of the estramustine phosphate.

11. A formulation according to any one of the preceding claims which is in single infusion dosage form comprising at least 950 mg/m^2 of the estramustine phosphate.

12. A formulation according to claim 10 or 11 wherein the basic amino acid is arginine.

13. A formulation according to any one of the preceding claims for intravenous use.

14. A formulation according to any one of the preceding claims for use in the treatment of cancer.

15. A formulation as claimed in claim 14 wherein the cancer is prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.

16. A formulation according to claim 1 wherein the parenterally acceptable carrier is a physiological solution for parenteral use which contains the basic amino acid, and the estramustine phosphate is in lyophilised form.

17. A product which comprises:

(i) a pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent and estramustine phosphate and a basic amino acid, and

(ii) one or more chemotherapeutic agents,

as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

18. A product according to claim 17 wherein the basic amino acid is arginine.

19. A product according to claim 17 or 18 wherein the chemotherapeutic agent is selected from the group consisting of taxane derivatives such as paclitaxel and docetaxel; camptothecin and derivatives thereof such as CPT-11 and 9-amino-camptothecin; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin, alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives such as carboplatin and cisplatin; angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668; optionally within liposomal formulations thereof.

20. A product according to claim 17 for intravenous use.

21. A product according to claim 17 for use in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.

22. A formulation as defined in claim 13 for use in suppressing or reducing the side-effects associated with the intravenous administration of estramustine phosphate and pharmaceutically acceptable salts thereof.

23. A formulation according to claim 22 wherein the side effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

24. A product which comprises estramustine phosphate in lyophilised form and a physiological solution for parenteral use containing a basic amino acid.

25. Use, in the manufacture of a medicament for parenteral administration, of estramustine phosphate and a basic amino acid.

26. Use according to claim 25 wherein the medicament is for intravenous administration.

27. A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, an antineoplastic agent known to cause ulcerative damages at the site of injection upon intravenous administration, and arginine or a pharmaceutically acceptable salt thereof.

28. A formulation according to claim 27 wherein the antineoplastic agent is selected from the group consisting of anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

29. Use of arginine, or of pharmaceutically acceptable salts thereof, in the preparation of a medicament for the treatment and prevention of side-effects associated with the intravenous administration of antineoplastic agents.

30. Use according to claim 29 wherein the side-effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

31. Use according to claim 29 wherein the antineoplastic agent is selected from the group consisting of estramustine phosphate; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

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(71) Applicant *(for all designated States except US)*: **PHARMACIA & UPJOHN S.P.A.** [IT/IT]; Via Robert Koch, 1.2, I-20152 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MUGGETTI, Lorena** [IT/IT]; Via Trento, 38, I-20036 Meda (IT). **COLOMBO, Paolo** [IT/IT]; Via Ludovico Cavaleri, 3, I-20147 Milan (IT). **MARTINI, Alessandro** [IT/IT]; Via Desiderio da Settignano, 14, I-20149 Milan (IT). **BUZZI, Giovanni** [IT/IT]; Via F. Poggi, 14, I-20131 Milan (IT).

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(54) Title: FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE AND AMINO ACIDS

(57) Abstract: A pharmaceutical formulations which comprises a parenterally acceptable carrier or diluent and estramustine phosphate and a basic amino acid. The formulation can be administered according to a combined chemotherapy regimen in association with one or more chemotherapeutic agents. The formulation also enables the estramustine phosphate to be administered with no side effects at the site of injection.

Declaration, Power Of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE AND AMINO ACIDS

the specification of which

- ☐ is attached hereto.
- ☐ was filed on 13/09/2000 as
  Application Serial No. PCT/EP00/08983
  and amended on _____.
- ☐ was filed as PCT international application
  Number _____
  on _____,
  and was amended under PCT Article 19
  on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
9921960.2	Great Britain	16.09.1999	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	**Filing Date**	**Status (pending, patented, abandoned)**

And we (I) hereby appoint the following registered practitioner(s):

022850

as our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to

022850

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-0d

Lorena Muggetti
NAME OF FIRST SOLE INVENTOR

Residence: Via Trento, 38
20036 Meda (Milan)
ITX

Signature of Inventor

Citizen of: Italy

Mailing Address: The same as above

4 March 2002
Date

2-00

Paolo Colombo
NAME OF SECOND JOINT INVENTOR

Paolo Colombo
Signature of Inventor

4 March 2002
Date

Residence: Via Ludovico Cavaleri, 3
20147 Milan (Italy)
TIX

Citizen of: Italy

Post Office Address: The same as above

3-00

Alessandro Martini
NAME OF THIRD JOINT INVENTOR

Alessandro Martini
Signature of Inventor

4 March 2002
Date

Residence: Via Desiderio da Settignano, 14
20149 Milan (Italy)
TIX

Citizen of: Italy

Post Office Address: The same as above

4-00

Giovanni Buzzi
NAME OF FOURTH JOINT INVENTOR

Signature of Inventor

4 March 2002
Date

Residence: Via F.Poggi, 14
20131 Milan (Italy)
TIX

Citizen of: Italy

Post Office Address: The same as above

NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date

Residence:

Citizen of:

Post Office Address: